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BARD Project Number: US-4128-08F

Date of Submission of the report: March 17, 2010

Project Title: Genetic Linkage Mapping of Basil (*Ocimum basilicum*)

Investigators	**Institutions**
Principal Investigator (PI): Faith Belanger	**Rutgers University**
Co-Principal Investigator (Co-PI): Nativ Dudai	**Agricultural Research Organization**
Collaborating Investigators: James Simon	**Rutgers University**
Nurit Katzir	**Agricultural Research Organization**

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order**:**

Budget: IS: $42,000 US: $58,000 Total: $100,000

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1			1
Submitted, in review, in preparation				
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant. Adolfina Koroch

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings				
Longer Visits (Sabbaticals)				

Description Cooperation:

N. Dudai provided plant material to F. Belanger and N. Katzir for assessment of gene-based marker development and AFLP marker development. N. Dudai developed the population and analyzed the F2 population for volatile compounds. F. Belanger and J. Simon collaborated on the analysis of nuclear DNA content in *Ociumum* species.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

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Abstract:

The ultimate goal of this project is to develop a genetic linkage map of basil (*Ocimum basilicum*). We received 1 year of funding from BARD to conduct a feasibility study. Below is a summary of our study. During this year we evaluated the cultivars 'Perrie' and 'Cardinal' for DNA sequence polymorphisms using AFLPs and gene-based markers. We evaluated an F2 population for variation in production of volatile compounds. We also determined the nuclear DNA content of 8 species of *Ocimum*. All of this information will be useful in the future for genetic linkage mapping of basil.

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Achievements:

The ultimate goal of this project is to develop a genetic linkage map of basil (*Ocimum basilicum*). We received 1 year of funding from BARD to conduct a feasibility study. Below is a summary of our study.

We focused on determining the level of variation between individuals from the basil cultivars 'Perrie' and 'Cardinal'. These cultivars were selected because they are quite different phenotypically and an F2 population developed from them was shown to be highly variable in morphology and chemical composition. We generated data for both AFLP markers and for gene-based markers.
The amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) is becoming one of the most popular methods in the fields of phylogenetic relationships, conservation and evolutionary genetics and ecology as it combines a high reproducibility and information content with the possibility of making genome wide screenings. AFLP technology has the capability to detect polymorphisms in different genomic regions simultaneously. AFLP markers are important in establishing linkage groups for placement of the gene-based markers, since many AFLP markers can be generated quickly. We analyzed four AFLP primer sets with DNA from the cultivars 'Perrie' and 'Cardinal' and a subset of the F2 population. This analysis yielded numerous polymorphic bands between the parental lines that are segregating in the F2.

We also evaluated polymorphisms in gene-based markers. Generating gene-based markers is more difficult and takes more time than the AFLP markers. However, for a linkage map to have wider utility than just the specific mapping population used to generate the map, it is critical to map genes. We focused on developing markers from COSII genes (Wu et al., 2006) since they are likely to be single copy genes and therefore easier to map. We have compared the 23,232 available basil ESTs with the 2871 COSII genes identified in Arabidopsis and found matches to 246. We screened 46 COSII genes from the available basil ESTs for polymorphisms. For nine of the COSII genes, polymorphisms were found between 'Perrie' and 'Cardinal'. Our screens were done using the dideoxy polymorphism scanning (ddPS) method (Rotter et al., 2007), which we used successfully to map colonial bentgrass (Rotter et al., 2009). Our standard procedure for marker development is to choose a COSII marker identified among the basil ESTs and do a BLAST search against the basil ESTs at NCBI to determine if other similar sequences have been reported. Alignments of similar sequences are made, and if there is a region of sequence variation detected, then PCR primers are designed to flank that region in an attempt to include variable regions in the PCR product. If there is no variation we still design PCR primers to amplify the 3' untranslated region of the EST.

We also analyzed 143 of the segregating F2 plants for essential oil composition. The essential oil of the cultivar 'Cardinal' is higher in methyl chavicol, while that of 'Perrie' is higher in eugenol. The essential oils of the F2 plants exhibited different combinations of phenylpropenes as well as other volatile compounds. Table 2 below illustrates the variation in some of the F2 plants.

Table 1. Essential oil composition of 'Perrie', 'Cardinal' and some F2 plants (% of total).

EO constituent	F2-4	F2-17	F2-102	F2-72	F2-135	F2-136	Perrie	Cardinal
1,8-Cineole	5.7	7.8	8.7	9.4	7.9	8.2	8.6	7.5
Fenchone	0.5	0.1	0.0	0.1	0.0	0.0	0.0	0.0
Linalool	16.8	20.1	20.8	19.9	17.8	21.4	15.5	14.2
1-Octen-3-yl-acetate	0.3	0.1	0.0	0.4	0.1	0.2	0.0	0.2
Fenchol	0.0	0.0	0.0	0.0	0.3	0.0	0.0	1.1
Borneol	0.4	0.4	0.3	0.2	0.3	0.2	0.4	0.5
□-Terpineole	0.4	0.6	0.7	0.8	0.6	0.8	0.8	0.5
Methyl chavicol	51.3	23.3	20.5	28.0	22.3	0.0	0.0	50.4
Fenchyl acetate	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2
Linalyl acetate	0.0	0.3	0.0	0.4	0.1	0.8	0.0	0.1
Chavicol	0.8	10.3	15.8	6.2	12.8	0.2	0.1	1.8
Bornyl acetate	0.3	0.6	0.4	0.8	0.6	0.7	0.5	0.2
Bicycloelemene	0.3	0.4	1.0	0.5	0.9	0.9	0.6	0.4
Eugenol	0.6	1.0	1.9	2.1	2.1	35.5	33.1	0.5
□-Elemene	0.9	2.0	2.1	1.4	0.0	1.7	1.8	0.9
Methyl eugenol	0.0	0.0	0.0	0.1	0.6	0.1	0.0	0.1
□-Humulene	0.3	0.4	0.4	0.5	3.0	0.4	0.5	0.4
Bicyclogermacrene	0.4	0.5	1.3	0.7	1.2	1.2	0.8	0.6
□-Cadinene	1.0	2.0	2.0	2.4	2.2	1.9	1.2	1.1
□-Cadinol	2.0	3.9	3.8	4.7	4.4	3.8	2.9	2.2
Total %	83.1	75.7	82.0	80.7	78.7	80.9	68.7	83.9

We also screened the F2 population and found that Fusarium resistance is segregating 3:1 (resistant:susceptible).

We also completed an analysis of nuclear DNA content for several *Ocimum* spp. For mapping it is useful to know the genome size of the species. Prior to this study there were no reports of nuclear DNA content for any *Ocimum* spp. This information will also be useful for breeders in providing a rapid means of screening material before initiating a breeding project. We examined multiple accessions of 8 *Ocimum* spp. and found that 2C genome sizes range from 928 Mbp to 5515 Mbp. The large variation in size is likely due to ploidy level differences. This study has been successfully completed and a manuscript on this work accepted for publication in The Israel Journal of Plant Science (Koroch et al., 2010).

References

Koroch, A.R., W. Wang, T.P. Michael, N. Dudai, J.E. Simon, and F.C. (2010) Estimation of nuclear DNA content of cultivated *Ocimum* species by using flow cytometry. Israel Journal of Plant Sciences, Accepted for publication

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Rotter, D., S.E. Warnke, and F.C. Belanger. 2007. Dideoxy polymorphism scanning, a gene-based method for marker development for genetic linkage mapping. Mol Breeding 19:267-274.

Rotter D., K. Amundsen, S.A Bonos, W.A. Meyer, S.E. Warnke, and F.C. Belanger. 2009. Molecular genetic linkage map for allotetraploid colonial bentgrass. Crop Science, 49: 1609-1619

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Friters, J. Pot, J. Paleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nuc. Acids Res. 23, 4407-4414.

Wu F.N., L.A. Mueller, D. Crouzillat, V. Petiard, and S.D. Tanksley. 2006. Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: A test case in the euasterid plant clade. Genetics 174:1407-1420.

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Details of cooperation:

The project objectives were promoted as a result of this cooperation by combining the breeding expertise and population development of N. Dudai with the molecular biology expertise of F. Belanger.

Publication:

Koroch, A.R., W. Wang, T.P. Michael, N. Dudai, J.E. Simon, and F.C. (2010) Estimation of nuclear DNA content of cultivated *Ocimum* species by using flow cytometry. Israel Journal of Plant Sciences, Accepted for publication

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Copy of manuscript accepted for publication in Israel Journal of Plant Science